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I, KAY WARD, ACTING MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 1753 for a patent by BREWTECH CARLTON UNITED BREWERIES filed on 21 July 1999.

I further certify that the name of the applicant has been amended to CARLTON & UNITED BREWERIES LIMITED pursuant to the provisions of Section 104 of the Patents Act 1990.

WITNESS my hand this
Twenty-fourth day of August 2000

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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s):

BREWTECH CARLTON UNITED BREWERIES

Invention Title:

SOIL CONDITIONER AND FERTILIZER

The invention is described in the following statement:

Soil Conditioner and Fertilizer

Field of the Invention

The present invention relates to soil conditioners and fertilizers. In particular it relates to soil conditioners and fertilizers which utilize by-products from fermentation processes. It especially relates to soil conditioners and fertilizers which are made from spent grain liquor.

Background of the Invention

One of the most critical problems associated with intensive agricultural activities has been the degradation of the natural environment due to the overuse of chemical fertilizers. Research has shown that the potential problems associated with the overuse of chemical fertilizers may outweigh the benefits of increases in crop production. However, despite this, it has been predicted that "the use of chemical fertilizers must be expanded two- to threefold to maintain soil fertility and productivity in the developing countries over the next 25 years if the world is to feed itself." Dr Norman Borlaug Nobel Peace Prize recipient.

There are a number of reasons why increased use of chemical fertilizers has been predicted, including increased human population and the replacement of traditional foods with cereals like wheat. However, a more worrying reason is that yields from traditional agricultural lands are falling. Although the reason for this is not known, even more chemical fertilizer has usually been used in an attempt to reverse the trend. However, one factor that has been noted is that the amount of topsoil in these areas has steadily been declining, and that normal microbial activity is reduced.

One area of agriculture which traditionally utilizes large quantities of chemical fertilizers is monoculture crop production. Monoculture crops include

sugarcane, cereals and turf. Turf production especially uses large amounts of chemical fertilizers. Turf production, as typified in golf course, race course, public area and parkland management, involves the maintenance of the appropriate nutrient levels in the soil, management of the sub-soil structure, protection against invasive fungal diseases, and development of a turf bed that is appropriate for the application (Crockford, 1992). The protocols used often place heavy emphasis on the use of inorganic nutrients, the use of fungicides, and intense mechanical interventionist procedures to produce satisfactory long term results.

Another problem associated with turf production is the continual loss of N, P, K and other nutrients from grass clippings. The extensive use of sandy substrata limits the retention of added inorganic nutrients, as sand provides few binding sites for the adhesion of inorganics. The addition of fungicides and chemicals to control fungal infestations is common practice, and the lack of organic complexity leads to low cation exchange capacity (CEC), making it more likely that these inorganic nutrients can leak into the water table. In addition, the permeability of the subsoil means that water utilisation is inefficient and energy usage through irrigation system is relatively high.

The production of turf and other monoculture crops has, like many others, seen a decline in yield in recent years (Magarey, 1994). Usually this decline has been regarded as a result of disease rather than attributed to the use of chemical fertilizers, and has been dealt with in four ways:

1) Chemically, by the use of fumigation and fungicides;
2) By the use of disease-resistant plants;
3) By rotation or fallowing; and
4) Biological control (Weller, 1988).

While these approaches have not solved the problems of decline in yield, they have produced some

interesting observations. Many studies have suggested that root health and, to a lesser extent, soil organic matter levels, are the main contributing factors to the growth of various plants (Papavizas and Lumsden, 1980). Moreover, it has been seen that declining monoculture yield seems to occur when continuous cropping with a susceptible crop results in disease.

However, it has also been observed that in some instances the disease-causing pathogen may create a favourable environment for multiplication of other microorganisms which are its natural enemies. This can occur because an adequate food base for the progressive development of microorganisms antagonistic to the pathogen is produced.

In some soil, disease does not occur in susceptible host plants even though the pathogen is present or is introduced into the soil. Such soils are referred to as suppressive and have been reported for several fungal pathogens such as *Phytophthora* sp, *Fusarium* sp, *Gaeumanomyces* sp, and *Rhizoctonia* sp for example. Soils that are generally suppressive the suppressive character is probably due to the entire microflora acting as a break on the growth and propagation of the pathogen(s). Specific suppressiveness occurs when one or two organisms control the pathogen through specific mechanisms (Cook, 1993).

In the case of turf, it has been shown that the severing of the leaf tips during mowing may provide an entry point for fungi such as *Rhizoctonia* and *Fusarium* spp which then colonise and debilitate the plants (Spurr and Knudsen, 1985; Schisler and Slininger, 1994; Kahl, 1978). However, suppressive compost mixtures have been developed which can combat some of these effects.

It has been suggested that suppressiveness may also be due to the architecture of plant resistance mechanisms due to the accumulation of some particular chemical elicitor(s). This has been suggested as an important means of disease control in plants (Cartwright *et*

al., 1977; Schönbeck and Dehre, 1986). Barley can activate a number of resistance mechanisms in response to attempted penetration by powdery mildew. These include the development of papillae with fluorescent haloes (Thordal-Christensen *et al.*, 1988), accumulation of inorganic compounds (Kunoh and Ishizaki, 1976), increased peroxidase activity (Kerby and Somerville, 1989), formation of phenolics (Shiraishi *et al.*, 1989) and the synthesis of proteins that appear to be a response to the pathogen (Apel *et al.*, 1990; Bryngelsson and Green, 1989).

Glucans have also been shown to elicit an immune response in animals, crustaceans and plants. This may be due to the fact that glucans are present in the cell wall of many fungi, and attachment of exogenous glucan to plant receptor sites may mimic the attachment of pathogenic fungi. Growth factors may also stimulate plant protection mechanisms; see for example our earlier application WO97/02356 the entire disclosure of which is incorporated herein via reference. During vigorous growth, cell multiplication and extension of tissue provides greater access to invasive pathogens. This enhances the systems designed to cope with stress.

All of these observation have led researchers to conclude that enhancing the well-being of the rhizosphere may be as important as providing nutritional support for the plants through fertilization. In other words, crop yields may be reduced in those areas that utilize the most chemical fertilizers because chemical fertilizers do not provide adequate resources for the natural microflora and fauna to proliferate. Without such microorganisms there is a greater incidence of root disease and pathogenic infection. Accordingly, there is a need for an alternative to chemical fertilizers which increases crop production, while minimizing the degradation of the environment. In particular, there is a need to have an alternative which reduces the incidence of disease in plants, improves root health and improves the levels of organic matter in the

soil.

One alternative which has been actively pursued in recent times is organic fertilizers. Generally, organic fertilizers are more environmentally friendly, while still providing very good nutritional support for plants. Furthermore, it has been shown that organic fertilizers have an added benefit in that they are capable of promoting the growth of soil microbes. These microbes often produce antimicrobials which are capable of deterring the growth of non-beneficial soil fungi, thereby preventing diseases of vegetables and lawn grass.

Some microorganisms, like *Trichoderma* and *Mycorrhiza*, aid a plant's uptake of water and nutrients and stimulates its growth, while others assist in:

1. Decomposition of crop residues, manure and other organic material;
2. Retention of nutrients;
3. Nutrient recycling;
4. Biological control of root rot and parasitic nematodes;
5. Production of plant growth regulators; and
6. Soil structure and tilth.

A further benefit of using organic fertilizers is that they are generally made from industrial wastes or animal effluent. These wastes have, for many years, been a source of environmental pollution in their own right; however, as increased negative data have been obtained about the use of chemical fertilizers, the use of organic wastes to make organic fertilizers has increased. Unfortunately, not all organic wastes are useful as organic fertilizers, and the processes involved in turning these into fertilizers can be costly. Accordingly, while the use of organic fertilizers is increasing, and the need for such organic fertilizers is evident, an appropriate source of "cheap" raw material has not been found to date.

One source of cheap raw material is waste by-products from the brewing and fermentation processes. Some

attempts at utilizing these wastes have been undertaken in the past. Most of these utilize the solid residue, primarily spent grain. Those which use liquid waste usually require treatment steps with high energy or chemical input. For example, Japanese Patent No. JP75002901 by Takara Shugo Co. Ltd. describes the use of waste liquid from brewing. The waste liquid contained yeasts, nonfermentable sugars, proteins, organic acids, and potassium. However, the waste liquid obtained after fermentation of molasses was condensed, and the recovered material was calcined at 850°C. This process is not only energy-intensive; all of the protein, enzymes, plant hormones and naturally present microorganisms are destroyed by the fermentation and calcination processes, resulting in a fertilizer which is potentially no better than a "normal" chemical fertilizer. Indeed, such a preparation would have low levels of sugars, dextrins, proteins, and vitamins.

Accordingly, the present invention attempts to overcome or at least alleviate some of the problems associated with providing a cheap, organic fertilizer which not only provides adequate nutritional support for plants, but also encourages the proliferation of soil microorganisms, thereby improving soil condition.

Summary of the Invention

The present invention provides a fertilizer composition comprising malt extract or spent grain liquor.

Preferably, the malt extract or spent grain liquor comprises cations, free amino nitrogen, glucans, carbohydrates, sugars, amino acid peptides, and polyphenols. More preferably, the malt extract or spent grain liquor is a by-product of fermentation processes. Most preferably, the malt extract or spent grain liquor is a by-product of beer brewing.

The fertilizer composition may comprise soluble solution plus particulates. The soluble solution may contain simple sugars, free amino nitrogen predominantly in

the form of protein, glucans, vitamins and calcines. Preferably, these constituents are extracted from the malt extract or spent grain during the brewing process.

The particulates may contain protein and carbohydrates.

The fertilizer composition may either be a liquid or a solid.

Preferably, the fertilizer composition further comprises one or more bacteria and/or yeast. More preferably, the bacteria are a mixture of organisms selected from the group consisting of *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Nitrobacter spps*, *Azospirillium spp*, and *Derxia* spp. Most preferably, the composition further comprises yeast extract and/or a yeast cell wall preparation.

The bacteria may be added in liquid suspension, as immobilized pellets, or as a dried powdered mixture.

The skilled addressee will appreciate that the application of the fertilizer composition to the plants will preferably provide soluble foliar and root nutrients and stimulators or plant protection mechanisms. The particulates will provide a slow release source of nutrients. The bacteria are designed to enhance the soil condition and promote the degradation of dead plant material, including thatch, and to reduce the opportunity for plant damage through fungal attack.

The present invention further provides a method of improving plant growth, comprising the step of applying to said plant an effective amount of a fertilizer composition comprising malt extract or spent grain liquor.

The composition may be applied to plants by any procedure known in the art. Preferably, the composition is a liquid and is sprayed on to the plants.

Those of skill in the art will appreciate that the application of the fertilizer composition to the soil will promote the growth and viability of the bacterial population present in the composition and the existing soil

microflora.

Such persons will further appreciate that the fertilizer composition may provide protection of seeds, promote healthy development and result in higher rates of seed germination in primary industry applications compared to untreated soil.

The present invention further provides a method of improving soil condition, comprising the step of applying to said soil an effective amount of a fertilizer composition comprising malt extract or spent grain liquor.

The malt extract or spent grain liquor may be fortified with yeast extract and / or yeast cell wall material which supplements the vitamin, sugar, cation, protein and polypeptide, carbohydrate and glucan content of the mixture. The mixture could also be fortified with organic and inorganic substances from a variety of sources in order to achieve specified levels of inorganics and organics.

The present invention further provides a method of preventing or inhibiting fungal growth, comprising the step of applying to soil or plants an effective amount of a composition comprising malt extract or spent grain liquor.

While not wishing to be bound by any specific theory, the applicants believe that the fertilizer composition of the present invention provides good soil structure and function which improves carbon exchange capacity (CEC), and may allow for better uptake of nutrients by the plant.

The proposed effects on foliage and soil are summarised in Figure 1.

The peptides and growth factors in the fertilizer composition are absorbed by the foliage and any wounded tissue areas. The microflora on the foliage is increased to provide an antagonist biofilm which combats the spread and pathologenicity of fungi. The biofilm also assists the breakdown of decaying foliage immediately below the green, photo-active foliage, which is commonly referred to as the

thatch layer. The carbohydrates and proteins provide substrates for the microflora, as well as the root system. The particles and the complex biopolymers also provide charged nucleation sites which enhance CEC. The insoluble protein/carbohydrate particles provide a constant source of amino acids, peptides and sugars which are slowly released by chemical and enzymatic activity. All of these together enhance the soil suppressive character of soils towards turf pathogens while at the same time providing essential nutrients.

When the fertilizer composition is further supplemented with bacteria, the degradation of existing organic matter in the soil and the grass, particularly thatch at the air/soil interface, is encouraged. Bacterial species such as *Bacillus, Pediococcus, Lactobacilli* and *Pseudomonas* are all active against some forms of fungi, and can therefore promote healthy growth by limiting fungal attack. Bacteria, including the *Lactobacilli* and *Pediococci;* are known to inhibit the growth of slime moulds such as *Fusarum* during the malting process.

Derxia sp are bacteria which secrete large amounts of polysaccharide material which provide active sites for the entrapment of nutrients and other bacteria. *Nitrobacter* and *Azospirillum* sp may also be added, as they promote nitrogen fixation for the improvement of the nutrient status of the subsoil.

In a particularly preferred embodiment a mixture of *Derxia gramosa, Azobacter beijeriacki, Pseudomonas fluorescens, Bacillus thuringiensis* and *Bacillus subtilis* is used.

These bacteria grow and divide rapidly in medium prepared spent grain liquor. The bacteria are grown in liquid culture under micro-aerobic conditions. All the genera referred to above aggregate during the growth cycle, but at the time of harvest still exhibit >75% cell viability.

Throughout the description and claims of this

specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", means "including but not limited to" and is not intended to exclude other additives, components, integers or steps.

Brief Description of the Figures

Figure 1 illustrates the way in which the fertilizer composition of the invention provides nutrients, immunostimulants, and provides greater intra-microbial competitiveness to support healthier plant growth.

Figure 2 shows the effect of the fertilizer composition of the invention at different dosage rates on appearance of nursery plots of golf course green turf.

Figures 3a shows the effects of the fertilizer composition of the invention on the thatch layer content of nursery plots of golf green turf.

Figure 3b shows the differential change in thatch content of the plots following treatment with the fertilizer composition of the invention.

Figure 4 shows the results of a comparative analysis of the soil chemistry of the turf plugs taken following treatment of nursery plots of golf course green with the fertilizer composition of the invention.

Figure 5 shows the effect of the fertilizer composition of the invention on the soil chemistry of "sandy soil" at a turf farm.

Figure 6 shows the effect of the fertilizer composition of the invention on the soil chemistry of "loamy soil" at a turf farm.

Detailed Description of the Invention

The present invention will now be further described by way of reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above. While the majority of

the examples in this specification are directed towards turf production, this is a representative application only, and those skilled in the art will appreciate that the fertilizer composition may be applied to other monoculture crops, especially cereal, vegetable and sugar cane crops, as well as in ornamental, forestry or horticultural plant production.

The malt extract or spent grain liquor used in the examples is preferably obtained from the brewing process. However, similar by-products and natural products may also be used. For example, molasses, waste from suagr refining, digested vegetable and animal matter (blood, tissue and the like); milk, powdered milk, extracted grape skins, digested grain husks, waste from the malting process and potato waste, could all be used as the untreated product, or following enzymatic pretreatment or blending. It will be appreciated that a mixture of one or more such components could be used.

Example 1 Recovery of Spent Grain Liquor

Malt was milled and extracted with hot water in the usual way for beer brewing. This step relies on endogenous enzyme activity of the malt to break down starch into monosaccharides and dextrins. Insoluble material, including the spent grain, was separated from the Wort extract by filtration using a Lauter tun.

The free-flowing liquid (spent grain liquor) was recovered from the spent grain by coarse separation technology to yield a suspension that contains approximately 5-20% v/v of insoluble material. These particles are approximately <500 micron in diameter. This material was concentrated or dried to achieve a more readily handled product.

The spent grain liquor also contains cations, carbohydrates, sugars, amino acids, peptides, vitamin A, thiamine, riboflavin, niacin, vitamin B12, vitamin C, vitamin B6, gibberellins and polyphenols. The suspension

also contains fine material, mainly insoluble protein and carbohydrate. The spent grain liquor also contains a naturally occurring population of microorganisms, including *Lactobacilli* spp and *Pediococcus* spp, which are antagonistic to fungal pathogens.

The composition of a typical spent grain solution is shown in Table 1.

Table 1: Composition of Spent Grain Liquor

Fine solids;		
Total phosphorus	0.05%	(w/w)
Total potassium	0.1%	(w/w)
Total nitrogen	6.4 - 7%	(w/w)
Total protein (approx. W x 6.25)	~ 40%	(w/w)
Total carbohydrate (fermentable)	- 1%	(w/w)
Holding solution		
Total phosphorus	0.01%	(w/w)
Total potassium	0.03%	(w/w)
Total nitrogen	0.7%	(w/w)
Total protein (approx. W x 6.25)	4.4%	(w/w)
Total carbohydrate (fermentable)	4%	(w/w)

Example 2 Yeast Extract and Yeast Cell Wall Preparation

Commercially available yeast extract preparations may be used; however, suitable yeast extract were also produced by the following methods.

Yeast suspensions were adjusted to pH 12 with an appropriate amount of concentrated HCl or NaOH solution. Chloroform (3%v/v final) was added, and the volume was adjusted to give a packed cell volume of 200ml/L. The mixture was then agitated and heated to 45-50°C for 24 hours. The soluble material was recovered by centrifugation at 5,000g for 10 min. Other suitable means could be used, for example, filtration. The recovered material was then spray dried directly, or concentrated using a surface swept evaporator before spray drying. Alternatively, the entire suspension after the heating step was dried.

Washed yeast suspensions were also prepared by adjusting to pH 9 and volume to 200ml/L. Commercial protease (Pancreatin, Esperase at 0.2% dosage rates (v/v)) was added to the stirred suspension and allowed to incubate at 60°C for 6 hours, while the pH was maintained at >11 by periodic addition of NaOH solution.

Yeast cell wall preparations were made as described in Patent Application Number WO97/02356.

Example 3 Bacterial Preparation

Bacteria were prepared by standard procedures. *Bacillus subtilis* was maintained on agar slopes at 4°C; the original stock culture was stored at -40°C in 50% glycerol. A shake flask of *B. subtilis* was prepared by inoculating a loop into nutrient broth. The cells were added to 20 l of medium containing 1% malt extract, 0.1% yeast extract in 0.01M potassium phosphate buffer pH 6.5. The plastic fermenter vessel was a typical beer brewing container fitted with inlet and exit ports for gas, and sterile filters in-line; the container was sterilised using

metabisulfite; the reverse osmosis water was passed through a Gelman 0.2 micron filter prior to addition to the vessel; the nutrients were dissolved in 2 litres of water and autoclaved at 121°C for 15 min and then added to the vessel. The vessel was inoculated with the bacteria and held at 30°C in a constant temperature room for up to 3 days. The final cell density was typically 10^{10} cell/ml. Once the cells had entered stationary phase the vessel was shifted to a cold room at 4°C. The cells were allowed to settle out over about 24h, although faster recoveries were sometimes obtained by adding silica gel (5g /litre, pH of the culture medium adjusted to 5). The cells were recovered from the culture medium by standard procedures.

Derxia gramosa, Azobacter beijeriacki, Pseudomonas fluorescens and *Bacillus thuringiensis* were all grown in the same medium and under the same conditions as described for *B. subtilis*.

Example 4 Fertilizer Composition Manufacture

The composition was prepared by mixing the spent grain liquor preparation disclosed in Example 1 with a bacterial mixture. Approximately 10^8-10^{12} organisms of each organism described in Example 3 were inoculated into the spent grain liquor preparation. The composition was prepared by mixing 1 liter (Low dose) or 5 litres (High dose) of each bacterial suspension with 30 litres of spent grain liquor preparation. The Low dose recipe provides about 3 x 10^{12} of each organism per litre. The High dose is 5 times greater.

Example 5 Yeast Extract Supplementation

The spent grain liquor was fortified with yeast extract, containing peptides, sugars, nucleosides, and nucleotides by addition of yeast extract powder (2.5 kg dry weight/tonne).

It was also fortified with yeast cell wall material, which contains immunostimulants including

glucans, by the addition of cell wall glucan (1 kg dry weight/tonne) prepared according to the method disclosed in WO97/02356.

Example 6 The Effect of Nutrient Applications on the Multiplication of Soil Bacteria

Preliminary experiments were undertaken to determine if yeast extracts and carbohydrate solutions could be used as nutrient sources to promote bacterial growth in soils. Bent grass was obtained from a commercial supplier and grown under lights in a sand/soil mixture in polystyrene containers (19 x 22 x 10 cm). Grass samples were dosed with yeast extract and bacteria (*B. subtilis*) in various combinations, samples were assayed 10 days after the treatments. The results are shown in Table 2.

Table 2. Total bacterial counts and *Bacillus* counts in soils with or without added bacteria and/or added yeast extract.

Origin of soil sample	Total Count				*Bacillus* count			
	Sample 1		Sample 2		Sample 1		Sample 2	
	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-5}	10^{-6}
Grass - no added bacteria -no added yeast extract	200	32	80	19	72	12	26	8
Grass - added bacteria - no yeast extract	90	33	60	12	48	11	31	6
Grass - added bacteria - added yeast extract	TNC	200	TNC	160	TNC	160	TNC	100

TNC - too numerous to count.

Sample 1 and Sample 2 represent typical samples from 10 replicates. Initially the grass samples had comparable bacterial counts in the top 2sm subsoil stratum, about 10^7 cfu/g soil. If bacteria were added without yeast extract, there was little change in the soil bacterial population. If both yeast extract and bacteria were added at the same time, there was a significant increase in the cell number. The increase in bacterial population was due in part to an increase in the number of *Bacilli*. However, if the addition of yeast extract-containing solution was delayed by 5 days there was no significant increase after a further 5 days of incubation.

These data indicate that the viability of the added bacteria is dependent upon the addition of significant amounts of nutrients and vitamins in the yeast hydrolysate. Similarly, the addition of molasses to grass samples did not significantly alter the measured total microbial population. When molasses solution was added together with *Bacillus sp* there was an increase in the microbial population. However, the dramatic increase outlined in the table above required the addition of the yeast extract as well. Preliminary data also indicated that the presence of glucans enhanced the effect. For example, clarified yeast hydrolysate was not as effective as the unseparated digested yeast suspension. Substitution of spent grain liquor for molasses, which contains little soluble or insoluble protein, had an effect comparable to that of molasses plus yeast extract.

The condition of the turf patches after the addition of yeast extract or yeast extract plus molasses was considered luxuriant, and rated more highly when bacteria were also included in the dosing regime.

Example 7 Effect of Fertilizer Composition or Nursery Test Plot of Golf Course Turf

The fertilizer composition as described in Example 5 was applied to a test plot at Huntingdale Golf

Course, Melbourne, Australia. The nursery test plot consisted of commercial bent grass, which was not pre-soaked, was in good condition, and was mowed after 4 weeks of application. The trials were carried out in duplicate during January and February 1999, i.e during the hottest summer months. The individual trial plots had a $50m^2$ surface area in an open location. No fungicidal or fertiliser treatments were applied during the trial periods. The appearance was rated on a scale of 1 to 5, with 1 being brown, patchy appearance and 5 being a lush, dark green appearance. The watering and dosage were stopped after 6 weeks of regular weekly dosing plus watering. After 2 weeks the watering, but not the application of fertilizer composition was resumed.

The nursery test plot was divided into a control panel and 4 test plots, each of which was treated with a different amount of the fertilizer composition - 5, 10, 20 and 30 litres/$50m^2$ of turf. The untreated nursery test plot was in reasonable condition and had been mown, but was rated at 1. The fertilizer composition was administered by hand-held spray. Between 10 and 60 litres per $100m^2$ surface area of turf was applied, using approximately 50 litres of water as the dilutant and carrier feed to the spray. The applications commenced on the first day of the first week and thereafter were effected one week apart. Samples of the grass were removed, photographed and analysed chemically. The samples were analysed for i) appearance, ii) soil chemistry, iii) microbial flora.

Prior to application of the fertilizer composition the test area was uniform in appearance, and had a rating of 1. Shortly after the treatment commenced there was a noticeable greening of the area treated with the highest amount of the fertilizer composition, and thereafter of the other test areas in order of increasing dosage rate. Initially the turf was rather thin, and the thatch layer was very apparent as a brown background. Within three weeks, the treated areas were appearing

greener and thicker ie had a reduced brown background compared to the control area. This was particularly apparent after about a month of treatment. All the treated turf areas were greener and more luxuriant than the control. After mid-February the area was not watered and its appearance deteriorated; the green foliage appeared to die away and the underlying brown thatch area was very visible. Watering resumed later in February, and the appearance of the whole area began to improve, but it was very apparent that the turf in treated area and especially the high dosage area revived at a much faster rate and became significantly thicker and greener that the other areas.

These observations have been quantitated in Table 3 on the basis of a quality rating from 1 to 5.

Table 3-Results of Trial of Fertilizer Composition on Golf Course Nursery Test Plots

	Test Plots				
Date	Control	5L/50m^2	10L/50m^2	20L/50m^2	30L/50m^2
7/1/99	1	1	1	1	1
11/1/99	1	1	1	2	2
13/1/99	1	1	2	2	3
15/1/99	1	1	3	3	3
20/1/99	1	2	3	4	5
25/1/99	1	2	3	4	5
27/1/99	1	3	4	5	5
10/2/99	1	4	4	5	5
15/2/99	2	4	4	5	5
23/2/99	1	1	1	1	1
4/3/99	1	2	3	3	4

A 1 rating was assigned to the appearance of the test plot prior to any treatment. The highest rating of 5 was given to the grass plot that received a sustained treatment of 30 litres of fertilizer composition per 50m^2 of area.

The latter plot rapidly reached the 5 rating after the trials started, and maintained this rating until the watering maintenance was halted. The rating improved noticeably after the watering recommenced. The areas treated with lower dosage rates also increased in quality quite rapidly once watering was resumed, and after 5 weeks there was little difference between the plots receiving 20 and 30 litres/50m^2. However it was noticeable that during the recovery period after the watering had recommenced the higher dosed plot outperformed the others.

The thatch layer in the nursery plot extended down about 15mm below the foliage. It was not continuous but resembled a series of layers that were loosely connected together. As shown in Figures 3a and 3b, there was some indication that after treatment the layers shrank in size or thickness and that the soil content in this section tended to increase. However, the effect was not consistently observed when plugs were examined, and it was therefore not clear whether the application of the fertilizer composition had a significant effect on the reduction of the thatch layer over the short time period of the trial.

Example 8 Chemical Analysis of Samples from Nursery Plots

Turf plugs from representative plots of treated turf and control turf described in Example 7 were taken and sectioned cylindrical sections (40mm x 150mm) were taken from the trial plots and sectioned vertically into three sections referred to as top, middle and bottom. These sections were analysed for Cation Exchange Capacity (CEC), Total phosphorus (P), Total potassium (K), Total nitrogen (N) and Total organic carbon.

The CEC gave an estimate of the number of charged sites in the soil capable of binding cations. Higher the value means that the soil is more effective in the retention of nutrients. N, P and K have the usual meaning

and importance in relation to plant nutrition. Total organic carbon gave some indication of the accumulation of organic material together with carbon fixed as a result of microbial activity and propagation.

Figure 4 provides a chronological series of histograms for the application of 10L and 50L/50 m^2 of fertilizer composition compared to a control, and Table 4 presents the raw data. In figure 4 the days refer to days after treatment. The litres refers to the number of litres of fertilizer composition applied to 50 m^2 trial plot area. The fertilizer composition was applied every 7 days. The trial plot area was watered, but received no other fertilizer applications for the duration of the sampling period. Abbrevations: CEC (Cation Exchange Capacity, mequiv Na/100g); P (Phosphorus mg/kg); K (Potassium mg.kg); TN (Total Kjeldahl Nitrogen mgN/kg); TOC (Total Organic Carbon mg/kg).

The control sample data are presented in Figure 4 (left hand and descending series of histograms). These data show:

TN - over time the levels in the deeper section of the plugs declines (hatched bars, compare day zero data point with day 31);

CEC - values vary between 5-10 in the top layer to <5 in the deepest section of the plug. These values declined by the end of the trial;

TOC - is distributed more or less evenly at the start of the trial throughout the core samples, but decreased noticeably during the time of the trial; and

P and K values were very low and at the limit of detection (<50mg/kg soil).

The plot treated with 10 litres fertilizer composition/50m^2 (centre and descending series of histograms) shows:

TN - in all sections of the core samples from the treated areas was much higher compared to the controls;

CEC - values for the test samples were consistently >10 in the top sections of the core samples, and were also higher throughout the plug as compared to the controls;

TOC - appeared to increase after treatment, and more carbon appeared to accumulate in the soil substructure; and

P and K in the top layer of the core samples were noticeably higher in the test samples compared to the controls.

The plot treated with 30 litres fertilizer composition/50m^2 (right and descending series of histograms) show:

TN - increased compared to the control, although values were lower than those obtained with the 30L/50m^2 fertilizer composition dosage rate;

CEC - values were higher than in the control, especially within the core samples at depth;

TOC - appeared to be higher than in the controls; and

P and K were about the same as the values found with the

Table 4

Results of chemical analysis of turf plugs for nursery plots

Date	Time	Application	Section	CEC, as Na	CEC, dry	P (dry)	K (dry)	Nitrate (dry)	Nitrite (dry)	TN (dry)	Moisture	TOC
8/01/99	Initial	-	top	5.9	7.1	<50	<50	<5	<5	830	16.7	1.99
8/01/99	Initial	-	2nd	6.8	7.7	<50	<50	<5	<5	640	12	1.5
8/01/99	Initial	-	3rd	4.2	4.7	<50	<50	<5	<5	500	10.9	1.09
8/01/99	Initial	-	bottom	3.7	4.1	<50	<50	<5	<5	330	9.4	1.09
15/01/99	7 days	control	top	5.5	6.2	-	-	<5	<5	660	11	1.74
15/01/99	7 days	control	2nd	7.3	8.1	-	-	<5	<5	710	9.8	2.15
15/01/99	7 days	control	3rd	5.8	6.3	-	-	<5	<5	660	8.5	1.75
15/01/99	7 days	control	bottom	8.6	9.5	-	-	<5	<5	440	9.7	1.69
15/01/99	7 days	10L/50m^2	top	12	14.1	81	110	<5	<5	1500	14.9	3
15/01/99	7 days	10L/50m^2	mid	10	11.3	58	<50	<5	<5	1300	11.3	2.5
15/01/99	7 days	10L/50m^2	bottom	6.7	7.4	<50	<50	<5	<5	650	9.6	1.7
15/01/99	7 days	30L/50m^2	top	6.6	10.9	-	-	<5	<5	1400	39.4	3.27
15/01/99	7 days	30L/50m^2	2nd	6.3	6.9	-	-	<5	<5	730	9.3	1.79
15/01/99	7 days	30L/50m^2	3rd	6.8	7.6	-	-	<5	<5	780	10.6	1.83
15/01/99	7 days	30L/50m^2	bottom	9.7	11.0	-	-	<5	<5	1000	11.5	2.84

Table 4 Continued

Date	Time	Application	Section	CEC, as Na	CEC, dry	P (dry)	K (dry)	Nitrate (dry)	Nitrite (dry)	TN (dry)	Moisture	TOC
22/01/99	14 days	control	top	8.3	11.8	-	-	<5	<5	1200	29.9	2.61
22/01/99	14 days	control	2nd	7.5	8.1	-	-	<5	<5	670	7.6	1.81
22/01/99	14 days	control	3rd	6.8	7.4	-	-	<5	<5	610	7.9	1.82
22/01/99	14 days	control	bottom	6	6.4	-	-	<5	<5	480	6.5	1.48
22/01/99	14 days	10L/50m^2	top	12	13.4	60	<50	<5	<5	1100	10.6	2.1
22/01/99	14 days	10L/50m^2	mid	8	8.8	<50	<50	<5	<5	970	8.8	1.9
22/01/99	14 days	10L/50m^2	bottom	6.5	7.1	<50	<50	<5	<5	600	8.4	1.6
22/01/99	14 days	30L/50m^2	top	7.5	10.7	-	-	<5	<5	1200	29.9	3
22/01/99	14 days	30L/50m^2	2nd	7.2	8.2	-	-	<5	<5	790	12.2	2.06
22/01/99	14 days	30L/50m^2	3rd	9.1	10.2	-	-	<5	<5	880	10.9	2.67
22/01/99	14 days	30L/50m^2	bottom	5.3	5.9	-	-	<5	<5	490	9.5	1.29
29/01/99	21 days	control	top	6.4	7.2	<50	51	<5	<5	810	11.4	1.2
29/01/99	21 days	control	middle	5.3	5.6	<50	<50	<5	<5	400	5.2	1.1
29/01/99	21 days	control	bottom	4.5	4.7	<50	<50	<5	<5	250	4.9	0.51
29/01/99	21 days	10L/50m^2	top	11	13.0	<50	160	<5	<5	1200	15.1	2.5
29/01/99	21 days	10L/50m^2	middle	8.2	9.3	<50	<50	<5	<5	920	12.2	1.6
29/01/99	21 days	10L/50m^2	bottom	5.8	6.3	<50	<50	<5	<5	480	7.8	1.5
29/01/99	21 days	30L/50m^2	top	5	5.7	<50	50	<5	<5	960	12.5	2.1
29/01/99	21 days	30L/50m^2	middle	8.1	9.0	<50	<50	<5	<5	760	9.7	1.8
29/01/99	21 days	30L/50m^2	bottom	6.7	7.3	<50	<50	<5	<5	580	8.6	1.4
5/02/99	28 days	control	top	7	8.8	98	260	<5	<5	1300	20.9	1.8
5/02/99	28 days	control	middle	6.2	6.9	<50	<50	<5	<5	570	9.8	1.3
5/02/99	28 days	control	bottom	4.1	4.4	<50	<50	<5	<5	300	7.3	0.78
5/02/99	28 days	10L/50m^2	top	10	12.1	86	190	<5	<5	1300	17.4	1.9
5/02/99	28 days	10L/50m^2	middle	8.3	9.4	<50	50	<5	<5	800	11.9	2
5/02/99	28 days	10L/50m^2	bottom	5.7	6.2	<50	<50	<5	<5	310	7.8	0.91
5/02/99	28 days	30L/50m^2	top	10	12.7	320	670	<5	<5	1100	21	1.3
5/02/99	28 days	30L/50m^2	middle	14	15.9	<50	54	<5	<5	850	12	1.9
5/02/99	28 days	30L/50m^2	bottom	5.6	6.2	<50	<50	<5	<5	510	9.1	1.5

Table 4 continued

Time	Time	Application	Section	CEC, as Na	CEC, dry	P (dry)	K (dry)	Nitrate (dry)	Nitrite (dry)	TN (dry)	Moisture	TOC
8/02/99	31 days	control	top	8.7	9.5	64	120	<5	<5	720	8.3	0.88
8/02/99	31 days	control	middle	4.4	4.7	<50	<50	<5	<5	440	7.1	0.85
8/02/99	31 days	control	bottom	1.5	1.6	<50	<50	<5	<5	210	6.2	0.53
8/02/99	31 days	10L/50m^2	top	17	21.7	110	170	<5	<5	2100	21.7	2.2
8/02/99	31 days	10L/50m^2	middle	8.3	9.3	<50	<50	<5	<5	770	10.9	1.9
8/02/99	31 days	10L/50m^2	bottom	5.1	5.5	<50	<50	<5	<5	490	8.1	1.1
8/02/99	31 days	30L/50m^2	top	9	11.4	140	190	<5	<5	1300	20.8	2
8/02/99	31 days	30L/50m^2	middle	8.5	9.8	<50	<50	<5	<5	940	13.1	2.3
8/02/99	31 days	30L/50m^2	bottom	5.7	6.2	<50	<50	<5	<5	420	7.8	1.1
10/02/99	33 days	control	top	4	4.6	<50	94	<5	<5	650	13.1	1.2
10/02/99	33 days	control	middle	3.1	3.3	57	<50	<5	<5	340	6.3	0.81
10/02/99	33 days	control	bottom	1.6	1.7	60	<50	<5	<5	150	4.7	0.41
10/02/99	33 days	10L/50m^2	top	7.8	11.4	81	130	<5	<5	2100	31.7	2.1
10/02/99	33 days	10L/50m^2	middle	7.4	8.4	<50	<50	<5	<5	880	11.4	1.7
10/02/99	33 days	10L/50m^2	bottom	4.7	5.2	<50	<50	<5	<5	410	8.8	0.98
10/02/99	33 days	30L/50m^2	top	7.8	9.2	51	73	<5	<5	970	15.2	1.2
10/02/99	33 days	30L/50m^2	middle	3.1	3.3	<50	<50	<5	<5	360	6.4	0.88
10/02/99	33 days	30L/50m^2	bottom	2.9	3.2	55	<50	<5	<5	340	9	0.76
26/02/99	49 days	control	top	3.7	4.4	90	250	<5	<5	780	16.7	1
26/02/99	49 days	control	middle	2	2.2	<50	<50	<5	<5	450	8	1
26/02/99	49 days	control	bottom	0.8	0.8	>50	<50	<5	<5	300	4.4	0.8
26/02/99	49 days	10L/50m^2	top	-	-	-	-	-	-	-	-	-
26/02/99	49 days	10L/50m^2	middle	-	-	-	-	-	-	-	-	-
26/02/99	49 days	10L/50m^2	bottom	-	-	-	-	-	-	-	-	-
26/02/99	49 days	30L/50m^2	top	5	6.4	170	290	<5	<5	1700	22.4	0.8
26/02/99	49 days	30L/50m^2	middle	2.8	3.1	<50	<50	<5	<5	720	8.6	1.6
26/02/99	49 days	30L/50m^2	bottom	2.3	2.5	<50	<50	<5	<5	600	8	1.2

Table 4 continued

Time	Time	Application	Section	CEC, as Na	CEC, dry	P (dry)	K (dry)	Nitrate (dry)	Nitrite (dry)	TN (dry)	Moisture	TOC
4/03/99	55 days	control	top	3.2	4.1	110	210	<5	<5	1100	22.2	1.3
4/03/99	55 days	control	middle	0.9	1.0	<50	<50	<5	<5	490	6.3	1
4/03/99	55 days	control	bottom	0.9	0.9	<50	<50	<5	<5	420	5.2	0.9
4/03/99	55 days	10L/50m^2	top	-	-	-	-	-	-	-	-	-
4/03/99	55 days	10L/50m^2	middle	-	-	-	-	-	-	-	-	-
4/03/99	55 days	10L/50m^2	bottom	-	-	-	-	-	-	-	-	-
4/03/99	55 days	30L/50m^2	top	15	18.3	110	68	<5	<5	2000	18	1.1
4/03/99	55 days	30L/50m^2	middle	3.3	3.7	<50	<50	<5	<5	800	10	1.9
4/03/99	55 days	30L/50m^2	bottom	1	1.1	<50	<50	<5	<5	600	8.8	1.5
12/03/99	63 days	control	top	15	18.9	71	270	-	-	1100	20.8	0.9
12/03/99	63 days	control	middle	3	3.2	<50	<50	-	-	400	5.9	0.8
12/03/99	63 days	control	bottom	1.3	1.4	<50	<50	-	-	260	4	0.4
12/03/99	63 days	30L/50m^2	top	9.2	12.1	120	140	-	-	1600	23.9	1.2
12/03/99	63 days	30L/50m^2	middle	3.8	4.2	<50	<50	-	-	810	9.2	1.6
12/03/99	63 days	30L/50m^2	bottom	5.3	5.8	<50	<50	-	-	720	8.4	1.2
19/03/99	70 days	control	top	11	14.4	110	170	-	-	1900	23.7	1.9
19/03/99	70 days	control	middle	5.7	6.3	<50	<50	-	-	960	10.2	1.9
19/03/99	70 days	control	bottom	2	2.2	<50	<50	-	-	520	7.2	1
19/03/99	70 days	30L/50m^2	top	5.4	6.9	210	290	-	-	1800	21.2	2
19/03/99	70 days	30L/50m^2	middle	4	4.3	<50	<50	-	-	580	7.1	1.2
19/03/99	70 days	30L/50m^2	bottom	3.5	3.8	<50	<50	-	-	620	8	1.2
26/03/99	77 days	control	top	14	19.2	160	260	-	-	2800	27	1.8
26/03/99	77 days	control	middle	6.9	7.7	<50	<50	-	-	1100	10.1	2.1
26/03/99	77 days	control	bottom	2.7	2.9	<50	<50	-	-	410	6.9	0.8
26/03/99	77 days	30L/50m^2	top	12	17.0	170	170	-	-	1800	29.5	1.2
26/03/99	77 days	30L/50m^2	middle	6.8	7.7	<50	<50	-	-	910	11.9	1.9
26/03/99	77 days	30L/50m^2	bottom	6.3	7.2	<50	<50	-	-	890	13	1.8

controls.

Accordingly, it will be appreciated that the CEC after treatment with the fertilizer composition was markedly increased. The initial CEC value was about 7 mega equivalents/100g of soil. After treatment the CEC values rose, and in some cases values of 20 were recorded. Total N in the soil was about 800mg/Kg soil before treatment, while after treatment 2100mg/Kg were detected. Total organic carbon was about 2mg/Kg in the soil before treatment, while after treatment levels of up to 3mg/Kg were detected. Potassium and phosphorus levels in the control and test areas were approximately the same.

Example 9 Microbial Populations in Nursery Plots

Turf plugs for the necessary plots described in Example 7 were analysed for microbial content. Total microbial counts were obtained for soil samples taken at different depths within the core samples. Total bacterial counts were obtained by serial dilution and platting on to nutrient and selective agar. An approximate estimate of the number of bacilli, pseudomonads and moulds was also determined. These tests were carried out aerobically, so micro-aerobic and anaerobic organisms may not been detected. The results are summarized in Table 5.

Table 5

Total microbial counts in turf plugs for golf course nursery plots

Date	Time	Application	Section	Pseudomonas (cfu/g x 10^3)	Moulds (cfu/g x 10^3)	Bacillus (cfu/g x 10^3)	Total Plate Count (cfu/g x 10^3)
8/01/99	Initial	control	top	2500	0	0	5000
8/01/99	Initial	control	middle	115	0	0	175
8/01/99	Initial	control	bottom	60	0	0	190
8/01/99	Initial	10L/50m^2	top	3000	0	0	3000
8/01/99	Initial	10L/50m^2	middle	75	0	0	110
8/01/99	Initial	10L/50m^2	bottom	55	0	1	0
8/01/99	Initial	30L/50m^2	top	1900	0	1	3500
8/01/99	Initial	30L/50m^2	middle	35	0	1	0
8/01/99	Initial	30L/50m^2	bottom	125	0	0	1600
15/01/99	7 days	control	top	2000	0	0	5500
15/01/99	7 days	control	middle	140	0	0	0
15/01/99	7 days	control	bottom	150	0	0	1600
15/01/99	7 days	10L/50m^2	top	2350	0	0	14500
15/01/99	7 days	10L/50m^2	middle	75	0	0	500
15/01/99	7 days	10L/50m^2	bottom	85	0	0	6500
15/01/99	7 days	30L/50m^2	top	1250	0	0	8000
15/01/99	7 days	30L/50m^2	middle	105	0	0	350
15/01/99	7 days	30L/50m^2	bottom	90	0	0	175
22/01/99	14 days	control	top	230	0	0	400
22/01/99	14 days	control	middle	75	0	0	50
22/01/99	14 days	control	bottom	50	0	0	120
22/01/99	14 days	10L/50m^2	top	80	0	1	500
22/01/99	14 days	10L/50m^2	middle	20	0	0	100
22/01/99	14 days	10L/50m^2	bottom	90	0	0	250
22/01/99	14 days	30L/50m^2	top	105	0	0	1750
22/01/99	14 days	30L/50m^2	middle	45	0	0	175
22/01/99	14 days	30L/50m^2	bottom	0	0	0	150

Table 5 continued:

Time	Time	Application	Section	Pseudomonas (cfu/g x 10^3)	Moulds (cfu/g x 10^3)	Bacillus (cfu/g x 10^3)	Total Plate Count (cfu/g x 10^3)
29/01/99	21 days	control	top	225	0	0	1750
29/01/99	21 days	control	middle	0	0	0	70
29/01/99	21 days	control	bottom	0	0	0	0
29/01/99	21 days	10L/50m^2	top	0	5	0	1500
29/01/99	21 days	10L/50m^2	middle	10	0	0	850
29/01/99	21 days	10L/50m^2	bottom	10	0	0	100
29/01/99	21 days	30L/50m^2	top	100	0	0	5500
29/01/99	21 days	30L/50m^2	middle	15	0	1	0
29/01/99	21 days	30L/50m^2	bottom	0	0	0	150
5/02/99	28 days	control	top	55	0	0	4000
5/02/99	28 days	control	middle	0	0	0	200
5/02/99	28 days	control	bottom	0	0	0	300
5/02/99	28 days	10L/50m^2	top	85	0	1	150
5/02/99	28 days	10L/50m^2	middle	0	0	0	200
5/02/99	28 days	10L/50m^2	bottom	0	0	0	100
5/02/99	28 days	30L/50m^2	top	35	0	1	5000
5/02/99	28 days	30L/50m^2	middle	5	0	0	100
5/02/99	28 days	30L/50m^2	bottom	10	0	0	0
8/02/99	31 days	control	top	10	10	1	1000
8/02/99	31 days	control	middle	10	0	0	750
8/02/99	31 days	control	bottom	0	0	0	150
8/02/99	31 days	10L/50m^2	top	0	5	4	2750
8/02/99	31 days	10L/50m^2	middle	0	5	5	150
8/02/99	31 days	10L/50m^2	bottom	0	0	0	800
8/02/99	31 days	30L/50m^2	top	50	10	3	1500
8/02/99	31 days	30L/50m^2	middle	5	55	0	200
8/02/99	31 days	30L/50m^2	bottom	15	0	0	175
10/02/99	33 days	control	top	25	0	6	1200
10/02/99	33 days	control	middle	25	0	5	100
10/02/99	33 days	control	bottom	90	0	2	900
10/02/99	33 days	10L/50m^2	top	160	0	1	125
10/02/99	33 days	10L/50m^2	middle	70	0	1	240
10/02/99	33 days	10L/50m^2	bottom	70	0	0	160
10/02/99	33 days	30L/50m^2	top	90	0	3	3500
10/02/99	33 days	30L/50m^2	middle	10	0	3	100
10/02/99	33 days	30L/50m^2	bottom	45	0	2	400

Table 5 continued:

Time	Time	Application	Section	Pseudomonas (cfu/g x 10^3)	Moulds (cfu/g x 10^3)	Bacillus (cfu/g x 10^3)	Total Plate Count (cfu/g x 10^3)
26/02/99	49 days	control	top	65000	0	7	74000
26/02/99	49 days	control	middle	900	0	0	740
26/02/99	49 days	control	bottom	650	0	0	780
26/02/99	49 days	30L/50m^2	top	10000	0	2	13000
26/02/99	49 days	30L/50m^2	middle	340	0	0	500
26/02/99	49 days	30L/50m^2	bottom	200	0	0	450
4/03/99	55 days	control	top	1000	0	4	8000
4/03/99	55 days	control	middle	120	0	0	160
4/03/99	55 days	control	bottom	130	0	0	560
4/03/99	55 days	30L/50m^2	top	5000	0	5	9000
4/03/99	55 days	30L/50m^2	middle	210	0	0	290
4/03/99	55 days	30L/50m^2	bottom	250	0	0	260
12/03/99	63 days	control	top	6000	0	5	10000
12/03/99	63 days	control	middle	130	0	0	250
12/03/99	63 days	control	bottom	0	0	0	450
12/03/99	63 days	30L/50m^2	top	10000	0	5	16000
12/03/99	63 days	30L/50m^2	middle	0	0	1	60
12/03/99	63 days	30L/50m^2	bottom	0	0	0	120
19/03/99	70 days	control	top	3000	0	1	3000
19/03/99	70 days	control	middle	0	0	0	180
19/03/99	70 days	control	bottom	50	0	0	440
19/03/99	70 days	30L/50m^2	top	5000	0	2	18000
19/03/99	70 days	30L/50m^2	middle	3000	0	0	600
19/03/99	70 days	30L/50m^2	bottom	15000	0	0	8000
26/03/99	77 days	control	top	22000	0	0	70000
26/03/99	77 days	control	middle	1000	0	2	510
26/03/99	77 days	control	bottom	30000	0	9	18000
26/03/99	77 days	30L/50m^2	top	5000	0	2	23000
26/03/99	77 days	30L/50m^2	middle	1000	0	0	1000
26/03/99	77 days	30L/50m^2	bottom	15000	0	0	5000

Cfu/g: colony forming units per gram sample

Bacterial counts were variable, but there was a trend to higher total numbers of bacteria in the samples for plots treated with fertilizer composition compared to the untreated plot. *Bacilli* were present in some soil samples, but at quite low numbers, while *Pseudomonads* were found in most samples at similar levels in treated and control samples. Treatment did not appear to encourage the growth of moulds as virtually no moulds, were detected in any of the soil samples

Example 10 Turf Trials on Turf Farms

The effect of the fertilizer composition on the growth of freshly sown bent grass was investigated and compared to controls. Trials were conducted in duplicate on freshly sown bent grass varieties on 100m^2 surface area trial plots at a turf farm located 100k NE of Melbourne on a river flats area of good fertility. The effect of various application rates of the fertilizer composition on grass appearance and soil chemistry was compared to control areas that received no treatment other than watering. The trial areas were also compared with the appearance of the overall field, which received the farm's usual fertiliser regime during the course of the trials. The dosage rates were varied from 10-60 litres/100m^2 turf for 10 weeks. The appearance of the test plots was analysed at regular intervals, and scored from 1 to 5 as described in Example 7. These data are shown in Table 6.

Table 6

Effect of the Fertilizer Composition on Turf Farm Test Plots

Turf Farm Test Plots						
Days	Control	5L/50m^2	10L/50m^2	20L/50m^2	30L/50m^2	60L/100m^2
0	0	0	0	0	0	1
28	0	1	2	2	3	-
35	0	2	3	3	4	-
42	1	2	3	4	5	-
63	2	3	4	5	5	5

There was a noticeable improvement in the appearance of the turf for all the test plots as compared with the controls, and also compared with the rest of the fields. The difference became more marked as the trial continued. The rating of highest dosage rate plot appeared outstanding compared to the control and to the rest of the plot at the 8-week period. There was some suggestion that *Rhizoctonnia* was present in this field, and after 6 weeks the entire field was treated with a fungicide. The sharp demarcation between the 60L/100m^2 treatment zone and the control suggests that the fertilizer composition may have some anti-fungal activity. Anecdotal evidence at the turf farm strongly suggests that this is the case.

Soil samples were taken during the trials and analysed for CEC, K, P, N, and carbon. The trials were conducted in duplicate, and it was apparent from the analysis that the duplicate trials that were positioned in "strips" and in sequence covered two soil types. One (Figure 5) was sandy, while the other contained a large amount of loam (Figure 6). In the sandy soil, the CEC levels were <5, throughout the core samples, and N, K, P levels were in the range targeted by the farm. The addition of the fertilizer composition had some effect on these parameters, but the effect was marginal, and nutritionally, ie in terms of N, K, P, it seems unlikely that these changes would have greatly affected turf root nutrition. The second trial showed similar trends with slight changes in the gross nutritional/soil chemistry indices measured. However, these data did not indicate that the significant visual improvement in the grass would be attributed to these changes. The results suggest that the improved grass is yield probably achieved or a control of pathogens, or nutrient delivery via the foliage, or both.

A further trial was undertaken while these tests were under way. A section of the field which showed fairly poor appearance was selected, and the fertilizer

composition was applied to the maturing turf using the previously established weekly dosing method. There was a noticeable improvement in visual appearance. The causal nature of the change in appearance can unambiguously be ascribed to the application of the fertilizer composition. The cause of the effect seems likely to be due solely to nutritional effects. Thus it seems that the fertilizer composition may act by enhancing the "soil suppressiveness" of the soil.

Example 11 Effect of Fertilizer Composition on the Activity of Plant Pathogenic Fungi

Plant pathogenic strains of fungi, namely, X, Y and Z, were incubated at 30°C for X days. Various concentrations of fertilizer composition as described in Example 1 was applied to the fungi. The fungi was then incubated at 30°C for X days and the growth monitored.

Rhizoctoni sp, Fusarium sp, and Fusarium sp were identified by visual inspection of turf plots and by microscopic examination of underlying soil samples. Turf was treated with the fertiliser and the treated and untreated areas were scored for infected areas. The turf treated with the fertiliser recorded a significantly lower number of infected sites compared to the control (18% of the control).

Challenge tests in which plant pathogenic fungi (Rhizoctonia and Pythia) are applied to healthy grass varieties in controlled conditions, in combination with different dosage rates of fertiliser are continuing.

Example 12 Effect of Fertilizer Composition on Other Monocrops

The fertilizer composition as described in Example 5 was applied to a cane test plot of commercial sugar cane. The test plot was not pre-soaked, and was in good condition. The trials were carried out in duplicate. The individual trial plots had a 50m^2 surface area in an

open location. No fungicidal treatment or fertiliser treatments were applied during the trial periods. The appearance was rated on a 1 to 5 scale, with 1 being poor appearance and 5 being good appearance. After 6 weeks of regular weekly dosing the treatments were stopped. It was evident that the sugar cane grew better with the fertilizer composition than without.

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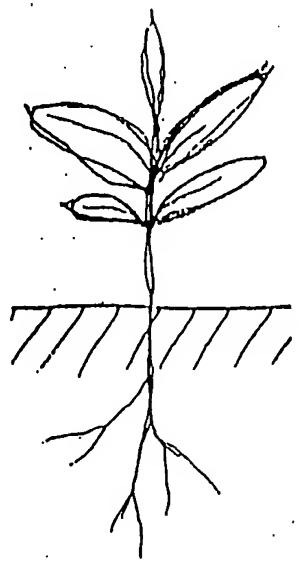


1. Peptide growth factors are absorbed by foliage and are accessible to cut tissue opening.
2. Microflora complexity on foliage is increased to provide an antagonistic biofilm to limit fungal spread.
3. Peptides, carbohydrates, growth factors are adsorbed to sub-soil particles and increase the soil cation exchange capacity.
4. Carbonates and peptides provide nutrients for multi plication / vitality of the microflora
5. Microflora develops soil suppressive character by natural means.
6. Immuno-stimulants including glucans attach to the root system and trigger immuno responsive cascade in the plants
7. Peptides and polysaccharides provide constant slow release of amino acids and sugars for plant root and microbial nutrition.

Figure 1

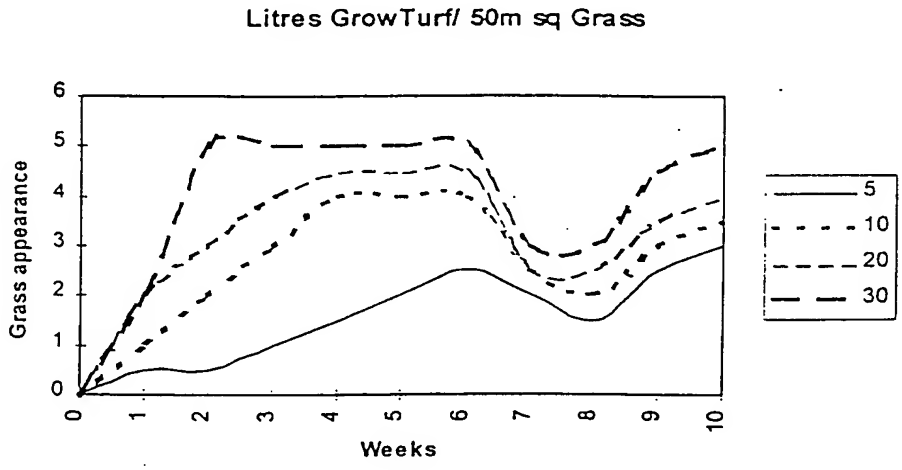

Litres GrowTurf/ 50m sq Grass
Grass appearance
0
1
2
3
4
5
6
0
1
2
3
4
5
6
7
8
9
10
Weeks
5
10
20
30

Figure 2

Figure 3a: The effect of BioTurf addition to golf green turf, on the observed thatch layer content.

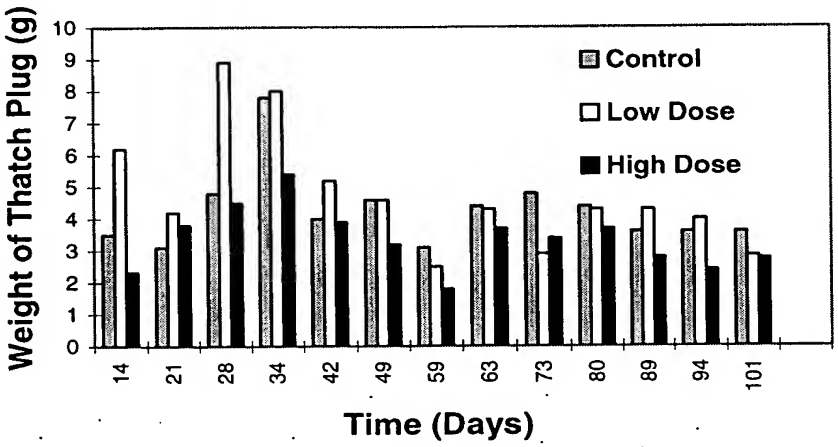


Figure 3b: Differential change in thatch content following treatment with two BioTurf formulations.

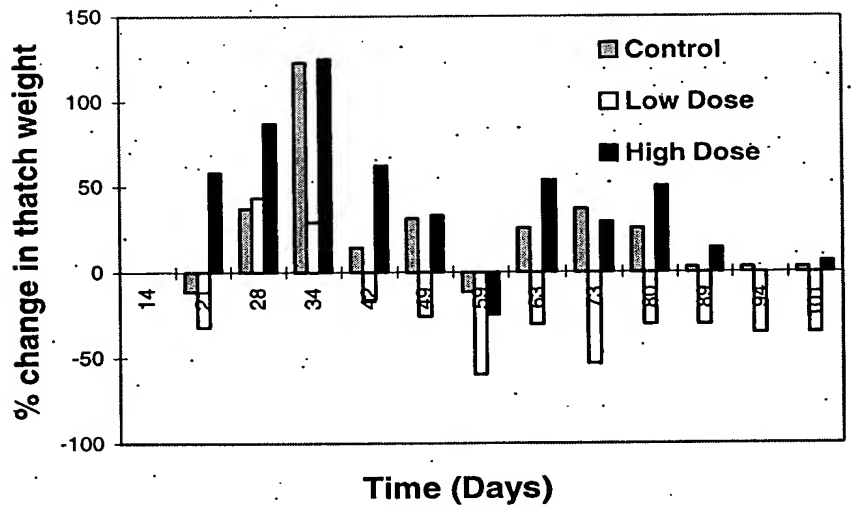


Figure 4: Comparative analysis of the soil chemistry following treatment of the Huntingdale Golf Course green with GrowTurf.

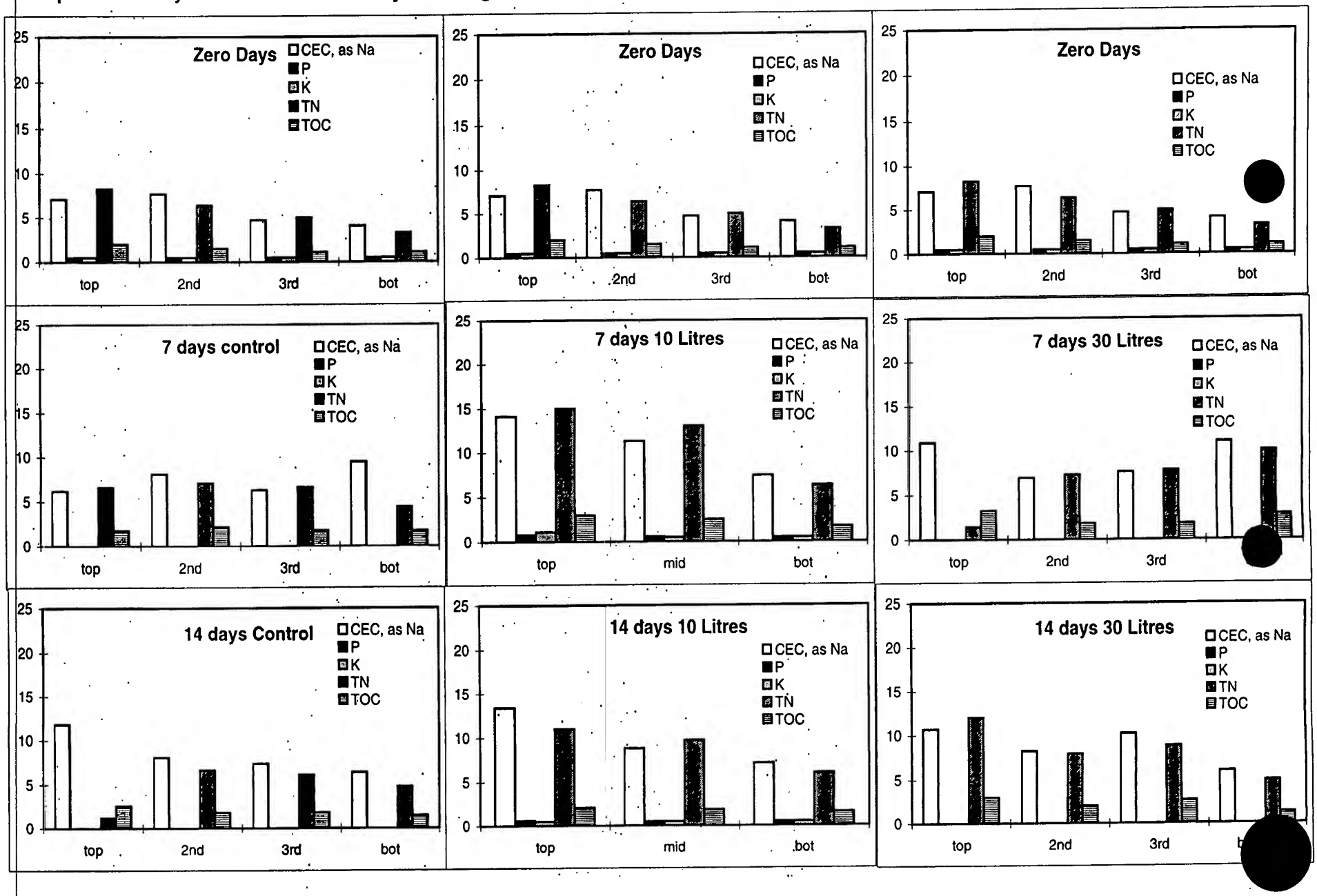


Figure 4: continued

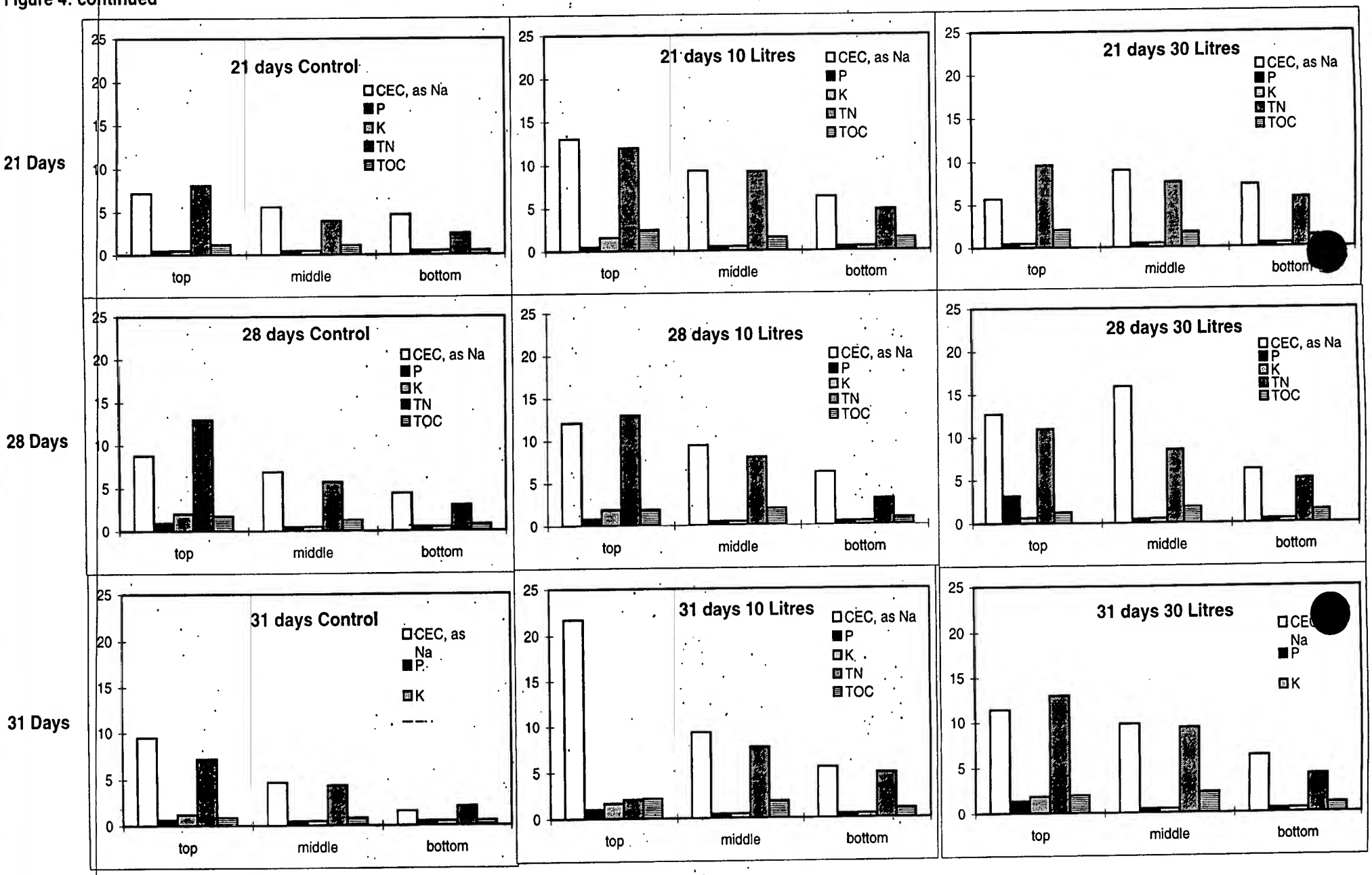


Figure 4: continued

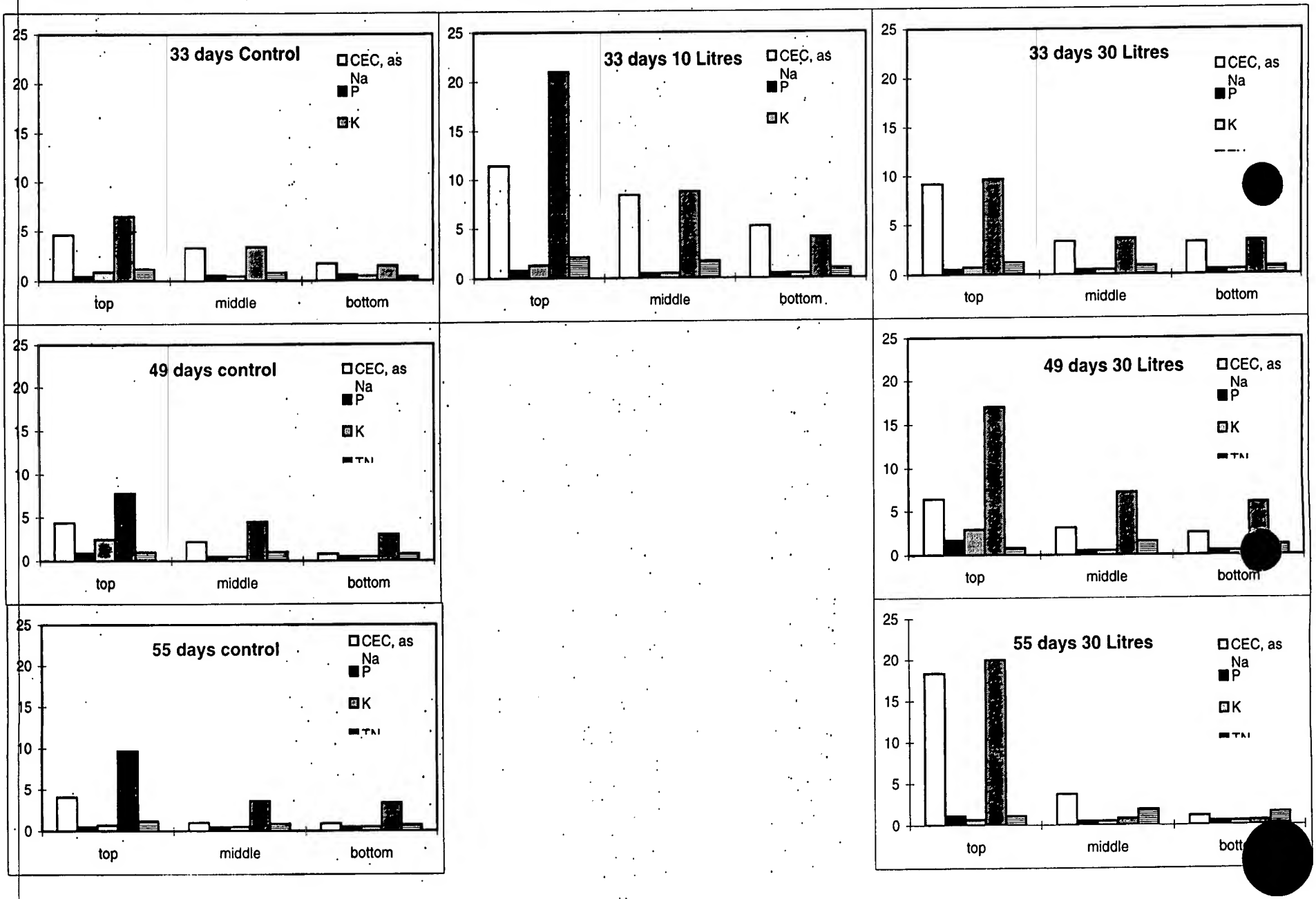
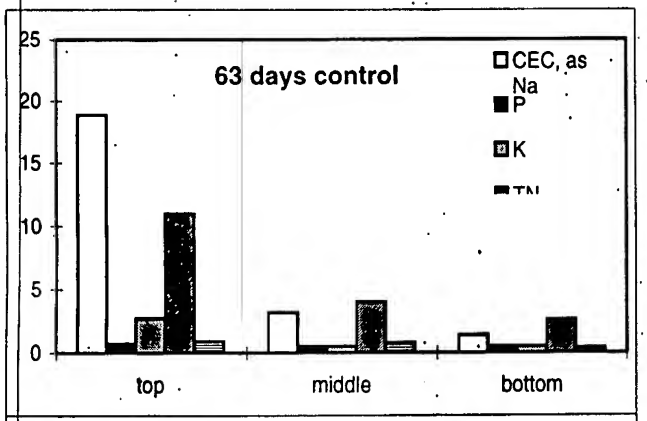
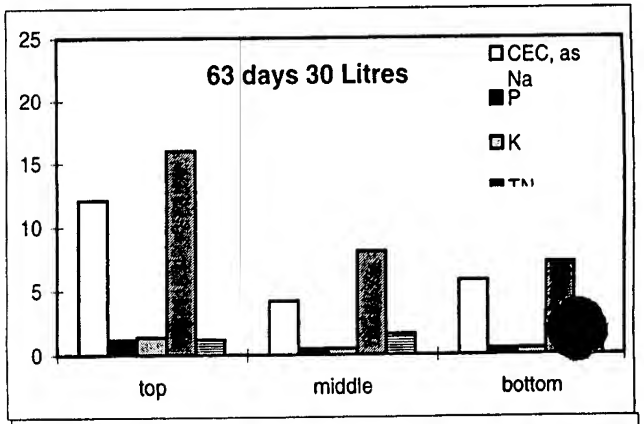


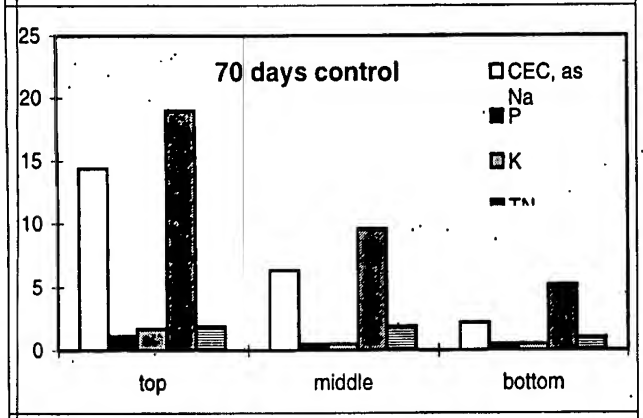
Figure 4: continued

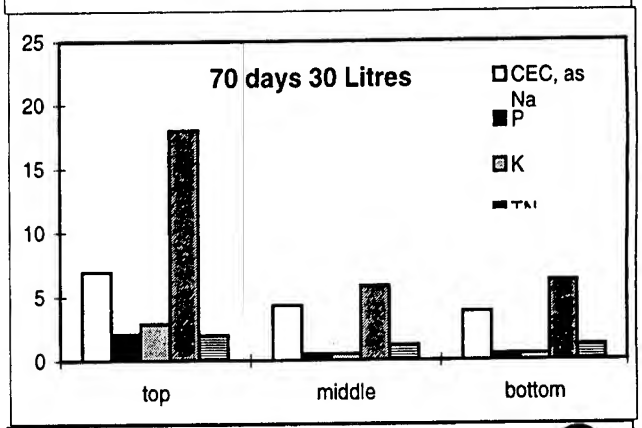
63 days



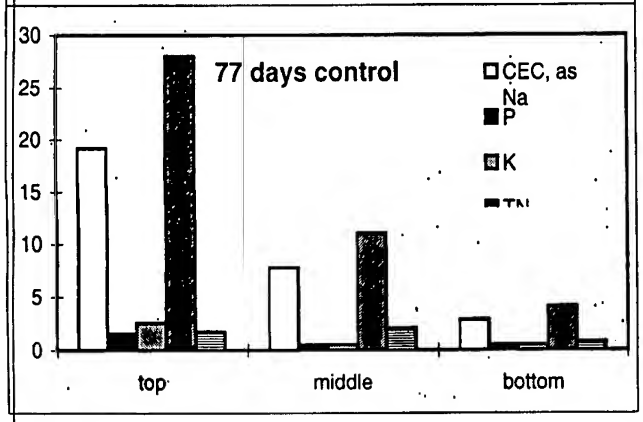


70 days





77 days



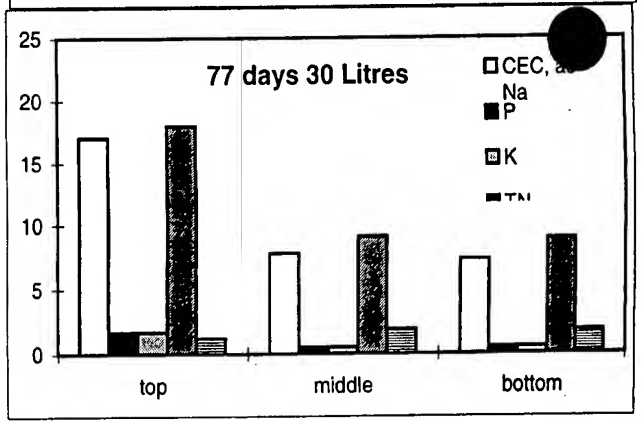
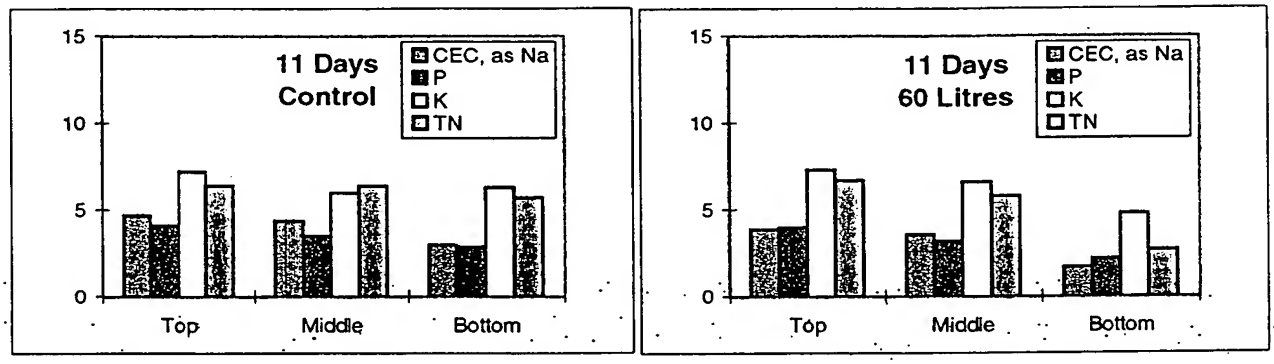
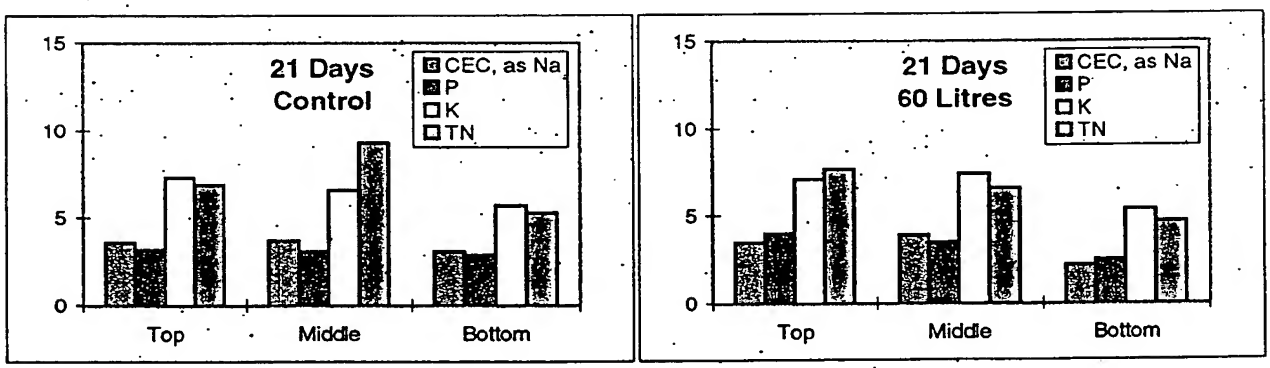


Figure 5: The effect of GrowTurf application on the soil chemistry at the StrathAyr Turf Farm. The data compares the control and the 60Litre GrowTurf / 100m^2 application zones for a typically 'sandy' soil area of paddock.

23rd March 1999, 11 Days



1st April 1999, 21 Days



8th April 1999, 28 Days

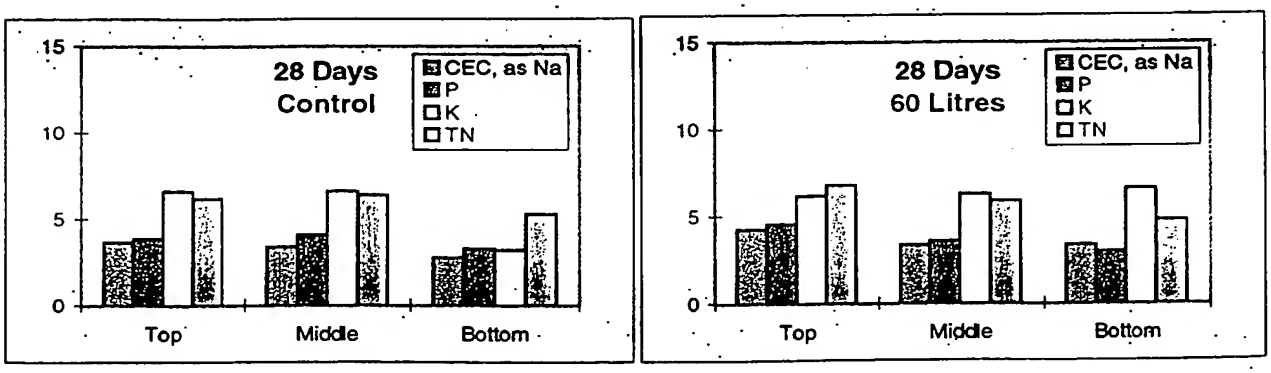
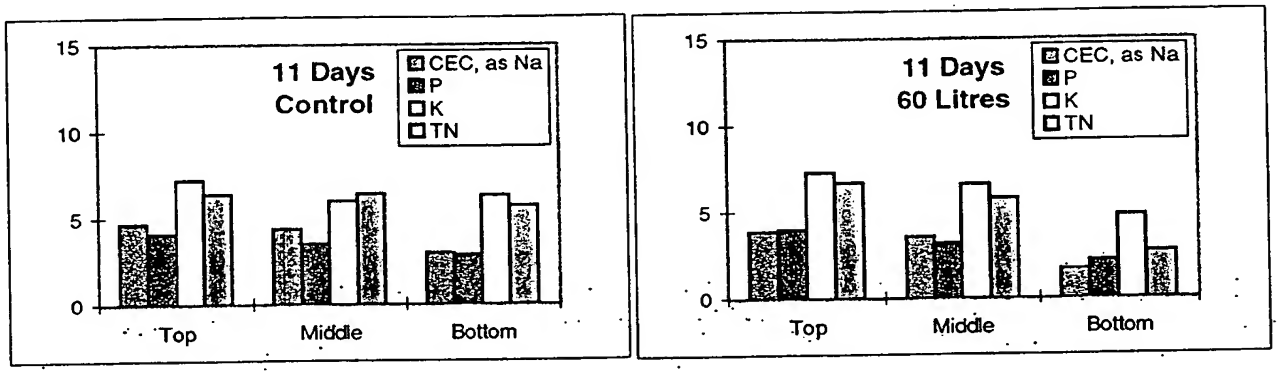


Figure 5: The effect of GrowTurf application on the soil chemistry at the StrathAyr Turf Farm. The data compares the control and the 60Litre GrowTurf / 100m^2 application zones for a typically 'sandy' soil area of paddock.

23rd March 1999, 11 Days



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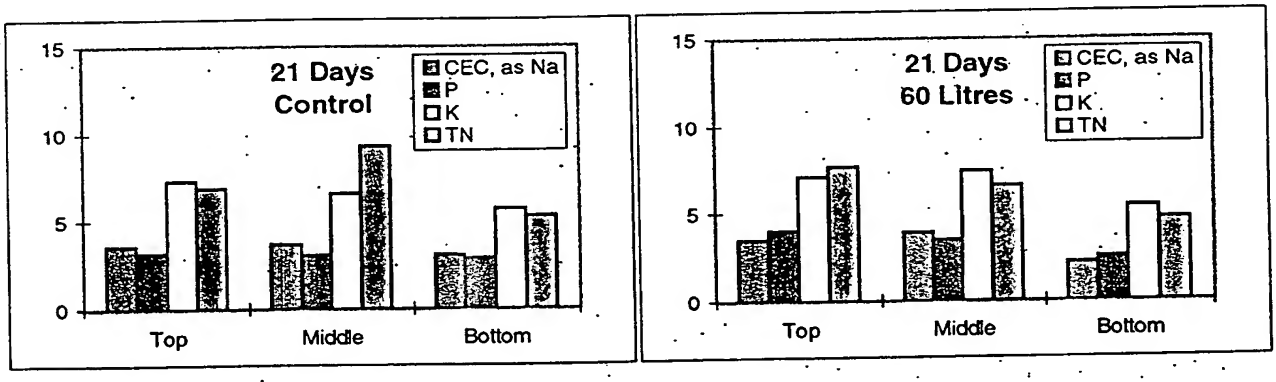

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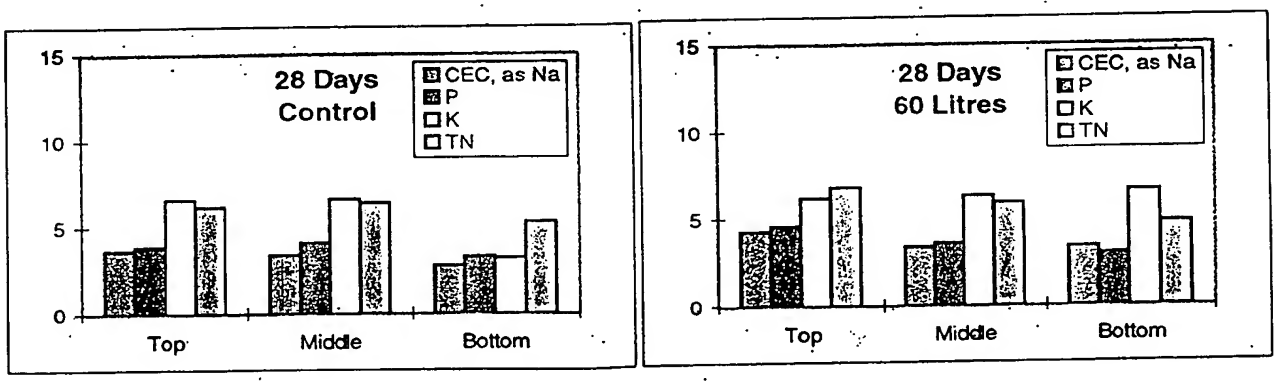
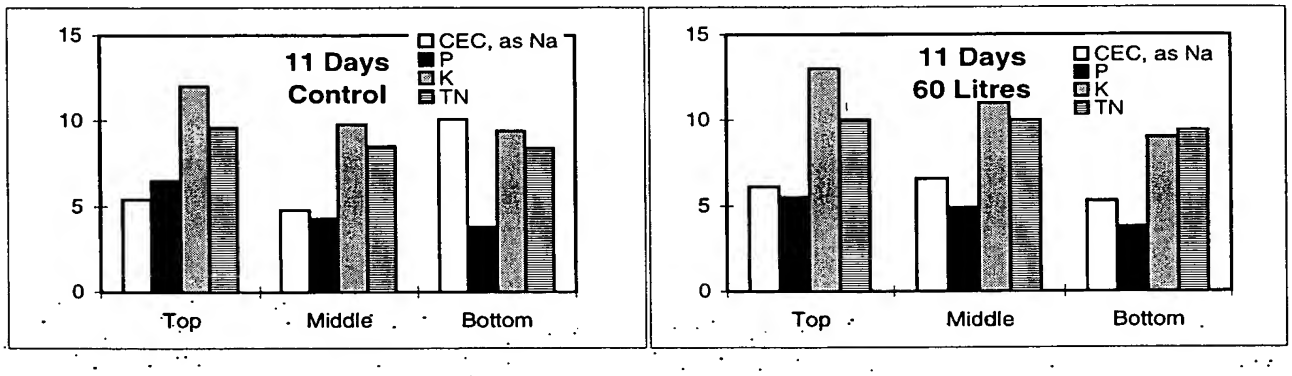


Figure 6: The effect of GrowTurf application on the soil chemistry at the StrathAyr Turf Farm. The data compares the control and the 60Litre GrowTurf / 100m^2 application zones for a typically 'loamy' soil area of paddock.

23rd March 1999, 11 Days



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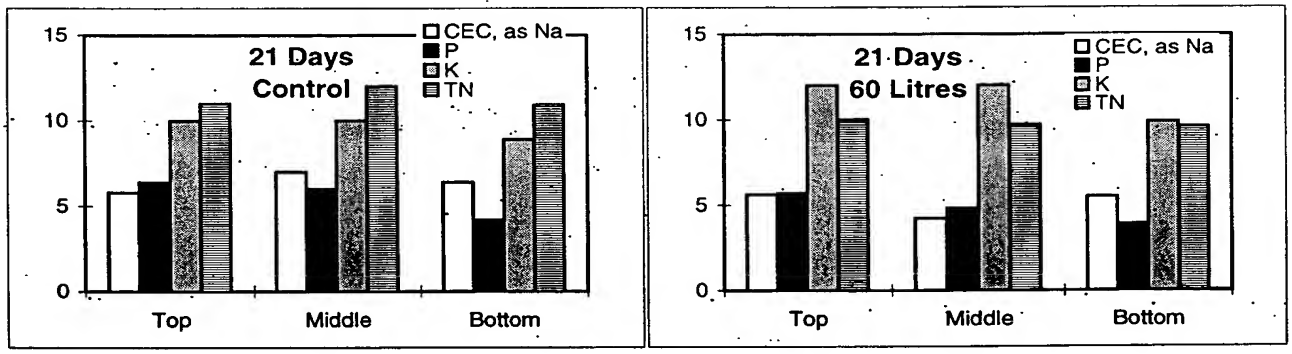

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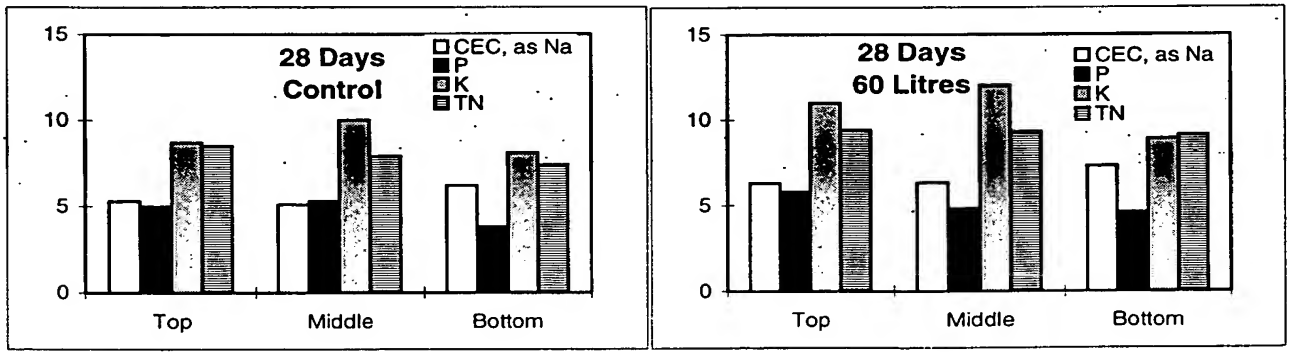
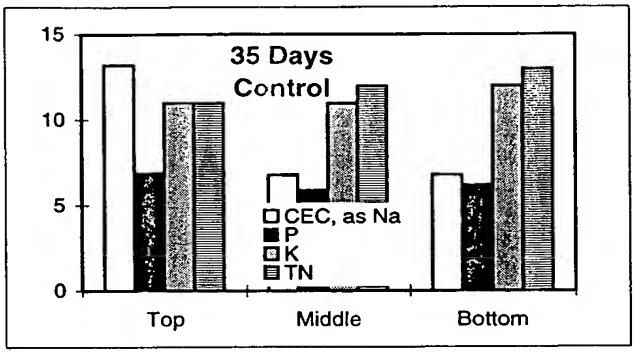
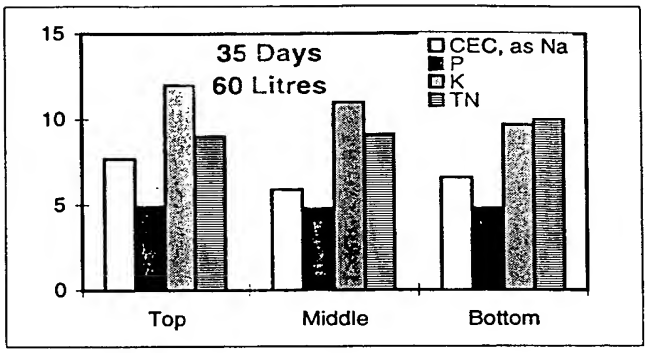


Figure 6 continued:

15th April 1999, 35 Days





22nd April 1999, 42 Days